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Physicochemical properties of cross linked acha (*digitaria exilis*) starch with citric acid

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ARTICLE INFO

Article type:

Research article

Article history:

Received April 2016

Accepted July 2016

April 2017 Issue

Keywords:

Crosslinking

Pasting viscosity

Emulsion capacity

Bulk density water

Oil absorption capacity

Least gelation concentration (LGC)

ABSTRACT

Acha (*Digitaria exilis*) starch was isolated and crosslinked using citric acid. Physicochemical properties including solubility, water and oil absorption capacities, bulk density, foam capacity, pasting viscosity and granule morphology were determined. Decrease in these properties, except for the emulsion capacity (from 36 to 38%) and least gelation concentration (LGC) which increased from 6% to 8% were observed with modified derivative. General decline was observed with water absorption capacity (488% to 465%); oil absorption capacity (122 to 116%); pH of starch slurry (6.85 to 6.45); bulk density (0.5 to 0.41g/ml) and foam capacity (4.0 to 3.0%). The starch granule morphology was investigated using scanning electron microscopy (SEM). Cross linking may not have affected the shape but appearance and structural arrangements of the starch granules differ upon modification. The starch granules retain its polygonal shape and the granule size ranges between 6um to 8.57 um. The infrared spectra of native acha starch and chemically modified derivative shown similar peaks, except for the additional peak in the cross-linked sample at 1614 cm^{-1} indicating the carbonyl bands stretching vibrations. Starch utilization as a food or feed relates to its physical and chemical properties. Cross linking altered these functional characteristics. Thus cross linking of Acha starch may find potential applications as good emulsifying agents, pharmaceutical excipients, disintegrants and drug carrier formulation.

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Capsule Summary: *Digitaria exilis* starch was crosslinked with citric acid and evaluated for solubility, water and oil absorption capacities, bulk density, foam capacity, pasting viscosity and granule morphology. Results revealed that Thus cross linking has significant impact on physic-chemical characteristics and product might be a potential a candidates as emulsifying agents, pharmaceutical excipients, disintegrants and drug carrier formulation.

Cite This Article As: S. Isah, A. A. Oshodi and V. N. Atasie. Physicochemical properties of cross linked acha (*digitaria exilis*) starch with citric acid. Chemistry International 3(2) (2017) 150-157.

INTRODUCTION

Unmodified starches generally are less undesirable for many industrial applications, because of their tendency to degrade under industrial processing conditions such as high

temperature, diverse pH, high shear rate, and freeze thaw variation (Musa et al., 2011).

Acha starch is a potential good binding material and good disintegrant for pharmaceutical applications (Musa et al., 2011).

A good starch produced today is from crops that serve as sources of staple foods. This development has necessitated research on alternative means of sourcing starch along with other products for domestic and industrial uses (Asif, 2015; Hussain et al., 2016; Iqbal et al., 2016a; Iqbal et al., 2012a; Iqbal et al., 2012b; Iqbal et al., 2016b; Iqbal et al., 2016c; Jamil et al., 2012; Mensah and Golomeke, 2015; Mensah et al., 2015; Mumtaz et al., 2016; Mushtaq et al., 2016; Naz et al., 2012; Perveen et al., 2011; Younis et al., 2015; Zia ul Haq et al., 2012). Focus on underutilized plants for starch production has stimulated research on crops such as mucuna beans, bambarra groundnut, new cocoyam, black Gram, Great Northern Bean, sago, pigeon pea, yam bean, field pea, lentil, Tiger nuts, Tacca tubers and jack beans (Emeje et al., 2012).

Acha, generally is underutilized. It provides cheap source of nutrients for some impoverished population and ready feeds for livestock. The starch is utilized in food industry, especially in biscuit factory as binder.

Unmodified native starch has its limitation in food and general industrial applications. Some of the disadvantages include:

- Loss of viscosity at low pH values, high processing temperature or mechanical treatment.
- Long texture
- Retrogradation-syneresis

Some reported studies on Acha starch focused on proximate chemical composition. This includes the work of Jideani and Akingbala (1993).

Since Acha is generally underutilized, and approximately 10% of its production provides cheap source of food for some impoverished population, there is need to get more findings and research on the quality of the starch, then improve on the quality through chemical modifications. This will serve as alternative source of starch than starches from staple foods and crops such as yams, corn, potatoes and plantain.

MATERIAL AND METHODS

Sample collection

White Acha (*digitaria exilis*) grains were purchased at a local market in Kubwa, Abuja, Nigeria.

The Acha sample was identified by the Biological sciences department, Bells University of Technology, Ota, Ogun State.

Preparation of starch slurry

The method described by Kunle et al (2003) with some modification was used. 2kg of winnowed D exillis was

steeped in 5 liters of distilled water for 24h at 28°C, after which the solution was discarded. The swollen grains was washed with water. The sample was then blended using a domestic milling machine. The slurry obtained was suspended in 5L of distilled water and then centrifuged at 4500 rpm for 30 minutes.

The starch was reslurried after centrifugation in 5L of distilled water and protein was separated from starch using 0.1M NaOH at PH of 8.5-9.0. An emulsion layer of denatured protein formed was discarded. The process was repeated for the starch slurry until the emulsion layer became less visible. The starch slurry was finally washed with acetone and air dried for 24hours at 28°C.

Starch cross-linking using citric acid

The method of Reddy and Yang (2009) with some modification was used for the cross linking of acha starch. 10g of native acha starch was suspended in 200 ml of distilled water to form a slurry. The starch dispersion was mixed thoroughly with 2g (20% w/v) of glycerol and heated to 80°C, and held at that temperature for 30minutes, then cooled to room temperature. To the starch dispersion was added 3g (30% w/w) of citric acid powder and stirred for 10 minutes. The starch solution was poured into casting container and left to dry for 72 hours. The cast films were peeled from the plates. Then the starch film was treated in a hot air oven at 165°C for one hour for the cross-linking reaction to occur. The film was finally transferred to an air tight container for future use.

Proximate Analysis of Starch and modified samples

Moisture content determination: The moisture content is expressed and recorded as percentage of the dried acha starch. 10g of acha starch were weighed on crucible. The samples and the crucibles were transferred to an oven maintained at a temperature of 105°C for 3 hours until a constant weight was obtained. Prior to weighing, the hot samples were transferred to a dessicator to cool for about 10 minutes.

The moisture content is calculated according to the following relation shown in Eq. 1.

$$\text{Moisture contents} = \left[\frac{c-a}{b-a} \right] \quad (1)$$

Where, a = initial weight of dish, b = initial weight of dish + sample, c = final weight of dish + sample.

Ash content determination: The dried material from (1) above was transferred to a muffle furnace. The porcelain dish in the muffle furnace was maintained at a temperature of 550°C for 24 hours. The dish now transferred to a dessicator and cooled. Ash content (total mineral constituents) is calculated using Eq. 2.

$$\text{Ash content} = \frac{\text{Ash Wt}}{\text{Sample Wt}} \times 100 \quad (2)$$

Determination of crude protein: 1gm of the sample was transferred to digestion tube and catalyst added. 15 cm³ conc. H₂SO₄ from dispenser was added and mixed carefully by swirling. The digestion tube was placed in the digester with a quick fit cap. Digestion was left for 2 hours until ready and exhaust cap removed and the sample cooled in the hood. The digest was now transferred to a distillation unit containing Boric acid and Sodium hydroxide and distilled for 5 minutes, after which the residue was titrated with 0.1M standard HCl to neutral grey. Finally the Nitrogen and crude protein was calculated using Eqs. 3 and 4, respectively.

$$N (\%) = 1.401 \times \left\{ \frac{\{\text{HCl titre (mL)} - \text{HCl blank}\} \times \text{MHCl} (\%)}{\text{sample wt}} \right\} \quad (3)$$

$$\text{Protein} (\%) = N (\%) \times 6.25 \quad (4)$$

Determination of crude fibre: Fat free residue was transferred from the soxhlet extractor into a 1 litre conical flask. 80ml of 1.25M H₂SO₄ was added into the conical flask and the whole content boiled for 30 minutes. The flask was rotated frequently and particles removed from the sides. The flask was removed after 30 minute and content filtered through Muslin Cloth. Washed with boiling water until the washing were no longer acidic to litmus. The residue was transferred back to the original digestion flask and brought to boil 80ml of the 1.25M NaOH and residue washed on the linen into the digestion flask with boiling NaOH. The reaction mixture was boiled for 30 minute while maintaining the Liquid level as above. Digestion flask was removed and filtered through the same linen cloth while the residue was washed with boiling water till free from Alkali. Residue was transferred to Gooch crucible, dried to constant weight at 105°C in an air oven. The gooch crucible and its content were transferred into a muffle furnace and ignited at 550°C until all carbonaceous matter were burnt. The crucible containing ash was cooled in a dessicator and weighed. The percentage crude fiber was calculated by subtracting the weighed ash from the weight obtained.

Determination of carbohydrate: The carbohydrate is made up of starch and sugar. This value was derived by deducting the total of crude protein, crude fat, crude fibre, total ash and moisture content from 100.

Extraction of sample (ether)

2gm of the dried sample was added into the extraction thimble of Soxhlet extractor and the thimble was carefully placed in the extractor. The weighed extraction flask was connected to the extractor carrying the thimble. Sufficient petroleum ether was poured into the extractor to start the

siphon which was then filled to about half the extraction flask. The vaporized and subsequently condensed solvent falls drop by drop into the thimble and the fat present in the food was extracted. When the level of solvent reaches the siphon height, the whole of the ether flows down into the flask leaving behind extracted fat which is then cooled in a desiccators and weighed. Continued drying, cooling and weighing was carried out until a constant weight not differing by more than 0.0002gm was obtained. The fat free residue was obtained for the subsequent crude fibre determination. The percentage of ether extract (FAT) was calculated as shown in Eq. 5.

$$\text{FAT} = \frac{\text{Increase in wt of flask}}{\text{Sample Wt}} \times 100 \quad (5)$$

Determination of physico-chemical properties

Solubility: The native starch and modified starch samples (2g each) were suspended in 20ml of distilled water and heated to 70°C for 30 minutes with continuous shaking. The mixture was centrifuged at 4000 rpm for 15 minutes. An aliquot of supernatant (5ml) was evaporated at 105°C and weighed. The solubility of starch is the ratio in mass (g) of the dried supernatant to the initial mass (g) of dried starch.

Water and Oil absorption capacity: 1g of native and modified starch was weighed into test tubes. 10ml of distilled water (and 10ml of groundnut oil in the second test tube) were added, and then heated in a water bath at 60°C for 30 minutes. The starch slurry was centrifuged at 1000rpm for 15 minutes and the supernatant carefully decanted and weighed and ratio was determined as shown in Eq. 6.

$$\frac{WAC}{OAC} = \frac{\text{Strach paste Wt}}{\text{Dry starch sample Wt}} \quad (6)$$

Bulk densities of native and modified starch: 2 grams each of native Acha starch and modified starch were placed in a 10ml measuring cylinder and the volume occupied by the sample without tapping recorded. The bulk density is the ratio of the weight to volume occupied.

The pH of starch: The pH of 1% w/v slurry of both the native starch and modified starch were determined using a pH meter.

Least gelation concentration of starch: 8 samples each for native and modified starches (1-16% w/v) were prepared in test tubes with 5ml of distilled water. The starch solutions were mixed using magnetic stirrer for 5 minutes and heated for 30 minutes at 80°C in a water bath followed by rapid cooling under running cold water. Further cool at 4°C for 2 hours. Least gelation concentration was determined as that conc. when the samples from the inverted test tube did not fall down or slip.

Table 1: Proximate composition of native and modified acha starch

Sample	Moisture (%)	Ash (%) Ether (%)	Fat Extract (%)	Crude fiber (%)	Protein (%)	CHO (%)
NAS	11.45±0.01	0.50±0.02	0.35±0.01	1.05±0.02	1.31±0.01	85.34
XAS	10.00±0.03	0.40±0.01	0.34±0.01	0.65±0.02	0.87±0.02	87.74

Table 2: Physico-chemical properties of native and modified acha starch

Sample	Solubility (g/100g)	Oil absorption capacity (%)	Water absorption capacity (%)	Bulk density (%)	Foam capacity (%)	Emulsion capacity (%)
NAS	10.24±0.01	122±0.02	488±0.01	0.5±0.02	4.0±0.01	36
XAS	8.03±0.03	116±0.01	465±0.01	0.4±0.02	3.0±0.02	38

Results are mean of triplicate determinations. NAS= native acha starch; XAS= cross linked acha starch

Pasting properties of the starch: The pasting properties of the native starch and modified starch was carried out using Viscometer.

Foam capacity of starch: 2 grams of native Acha starch and modified sample were homogenized in 100 ml of distilled water using a magnetic stirrer for 5 minutes. The homogenate was poured into a 250ml measuring cylinder and the volume occupied was recorded after 30 seconds. The foam capacity is expressed as the percent increase in volume.

Emulsion capacity of starch: 2 g of native and modified acha starch were dispersed in 25ml of distilled water using a magnetic stirrer for 30 seconds. After complete dispersion, 25ml of vegetable oil (groundnut oil) was added gradually and the mixing continued for another 30 seconds. The content was centrifuged at 1600rpm for 5 minutes and volume of oil separated from sample was read directly from the tube. Emulsion capacity is the amount of oil emulsified and held per gram of sample.

Starch Granules Morphology: Starch granule Morphology of both the native starch and modified starch were obtained using scanning electron microscopy (SEM).

RESULTS AND DISCUSSION

Proximate composition

The proximate composition of the native acha starch (NAS) and modified sample are presented in Table 1. The moisture content of the starches decreased from 11.45% to 10 % upon crosslinking. The ash content and ether extract (fat content) ranged from 0.50% to 0.40% and 0.35% to 0.34%

respectively. The protein content also decreased from 1.31% to 0.87%, while the crude fiber content decreased to 0.65% from 1.05%. The carbohydrate content increased from 85.34% to 87.74%.

These low values of moisture content following modification are advantageous especially in terms of shelf life and reduced spoilage under various storage conditions. This agreed with the results of Olu-owolabi et al (2013) and Emeje, et al (2012). Both native acha starch (NAS) and modified samples were dried under the same conditions. Therefore, the reduction of moisture content may be due to the substitution of the hydroxyl groups on the starch molecules.

The ash content also shown a similar pattern of reduction due to washing away of the mineral contents of the starches during modification such as, acetylation, etherification and oxidation processes. Adebowale et al (2002) reported that ash content of modified jack jean starch reduced considerably after modification and they reported this observation to washing away of the starches mineral contents. The crude fiber and ether extract (fat content) were also reduced following modification of the native acha starch. A decline in protein content was observed with the cross-linked sample The carbohydrate content following modifications increased from 85.34% to 87.74

Physico-chemical properties

Solubility: The results of solubility of native acha starch (NAS) and cross-linked starch (XAS) are shown on Table 2. The solubility expressed as gram per 100 gram of starch (g/100g) reduced from 10.24 value observed with the native acha starch to as low as 8.03g for cross linked sample (XAS). This decline in solubility following crosslinking may be attributed to decrease in the hydroxyl groups resulting from the break in glycosidic bond linkages, and also due to

Table 3: least gelation concentration

Concentration%	NAS	XAS
2	Viscous	Viscous
4	Viscous	Viscous
6	Gel	Viscous
8	Gel	Gel
10	Gel	Gel
12	Gel	Gel
14	Gel	Gel
16	Gel	Gel

Table 4: Pasting Viscosities of starch (Cp)

Sample	viscosity (Cp)
NAS	31.5
XAS	25

Results are mean of triplicate determinations. NAS= native acha starch; XAS= cross linked acha starch

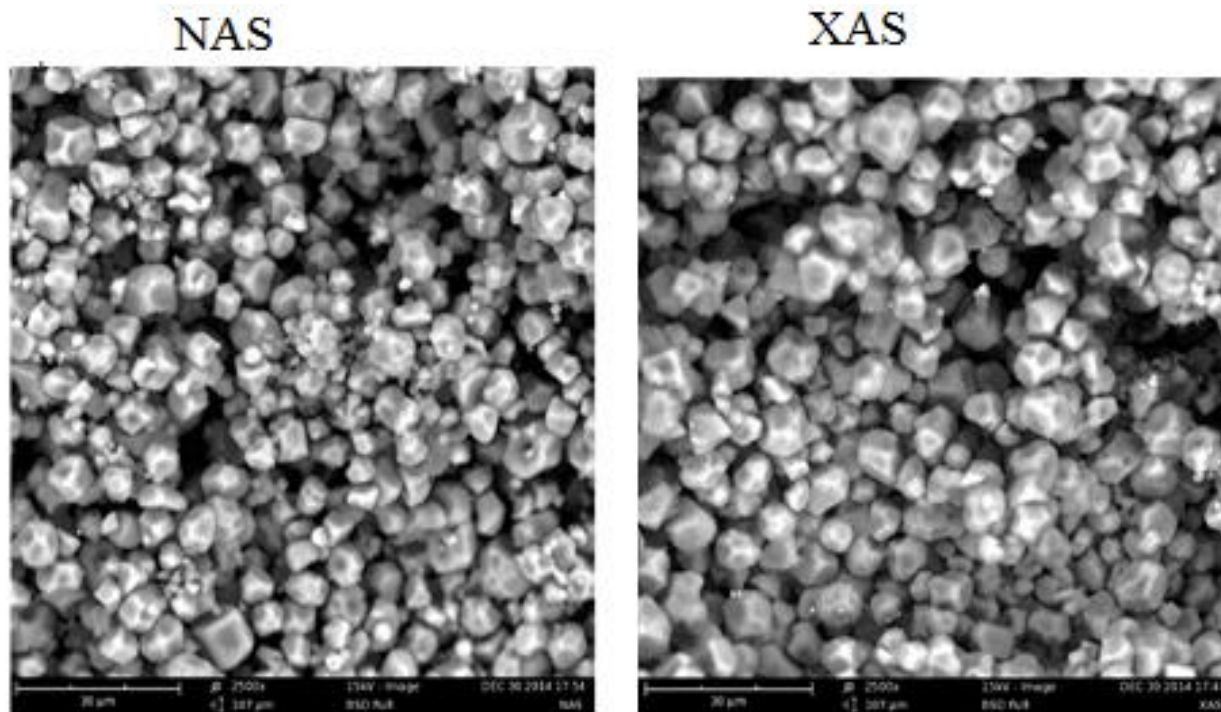
introduction of bulky functional groups reducing the mobility of starch molecules. This pattern agreed with the results of Olu-owolabi and Adebowale (2013).

Water absorption capacity: The water absorption capacity of native acha starch and chemically modified sample are presented in Table 2.

Water absorption capacity of native acha starch declined from 488% to 465%. This may be attributed to the incorporation of hydrophobic acetyl functional groups on the starch molecules which reduced water binding capacity more than the native starch. This result was in agreement with the observations reported on water absorption capacity of modified bambara groundnut starch by Adebowale, et al (2002).

Oil Absorption Capacity: The result of oil absorption capacity of native acha starch and modified sample are presented in table 2. A decline from 122% to 116% was observed. This may be attributed to the functional groups incorporated onto the starch molecule following chemical modification. This agrees with the report of Sathe and Salunkhe (1981) that acetylation and oxidation do not improve oil absorption capacity of great northern bean.

pH of starch slurry: The result of pH of starch slurries of native and modified acha starch shows a decline upon modification. The pH values decreased from 6.85 to 6.45 after modification. The reduction in pH values of acetylated sample may be attributed to the incorporation of acetyl functional

**Fig. 1:** Scanning electron Microscopy (SEM) of NAS and XAS

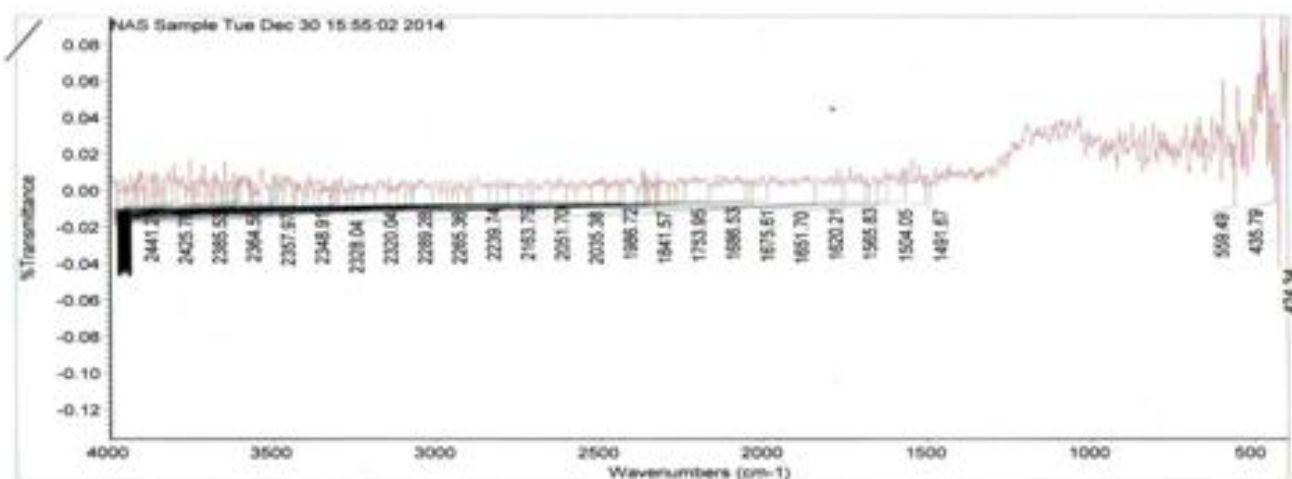


Fig. 2: Infra red spectra of NAS Sample

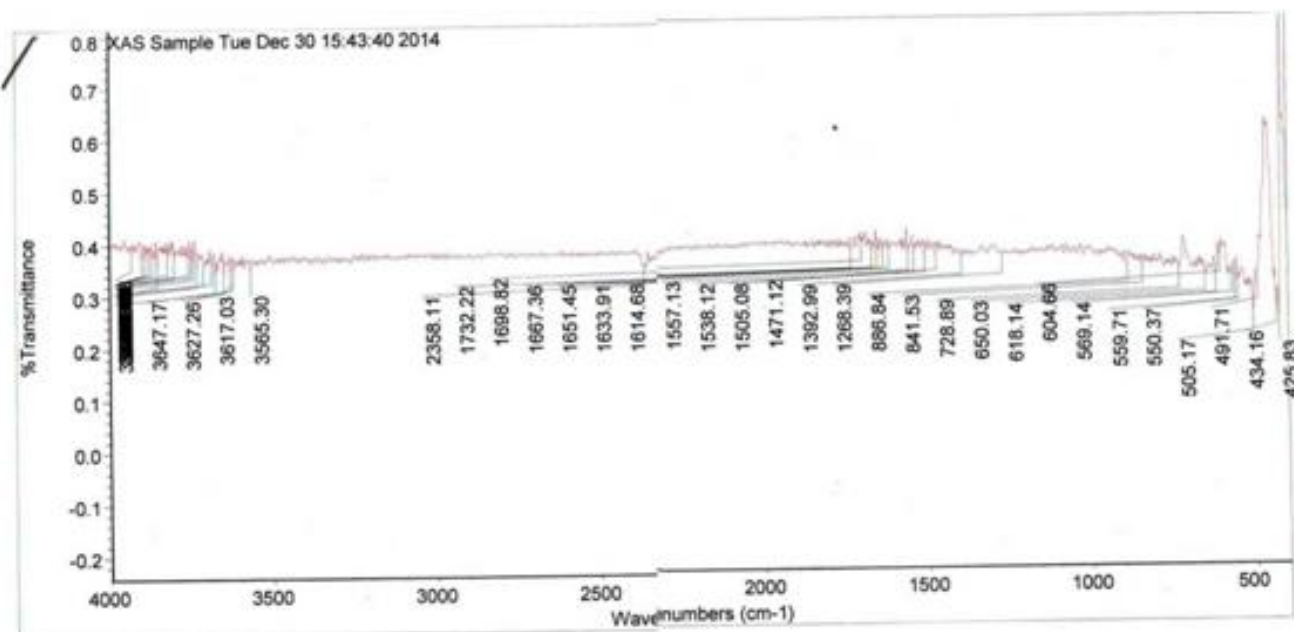


Fig. 3: Infra red spectra of XAS Sample

group to the starch molecule thereby increasing the acidity of starch molecules.

Bulk density: The bulk density of native acha starch and modified sample are presented on table 2. The bulk density declined from 0.5 to 0.41 g/ml due to increased crystallinity following chemical modification. Thus retrogradation of native acha starch as well as seneresis may be improved upon modification. This reduction in bulk density is in agreement with Emeji, et al (2012).

Foam capacity: The result of foam capacity of native acha starch and modified samples are presented on table 2. The foam capacity of acetylated (cross linked) was reduced to 3.0% from 4.0% . Reduction in foam capacity following

crosslinking could find application as an emulsifier in the food industries (Ihegwuagu, et al. 2008).

3.3.7 Emulsion capacity

The emulsion capacity of native acha starch and cross linked sample is presented on Table 2. The emulsion capacity increased from 36% to 38%. This suggests that chemically modified acha starch are better emulsifying agent due to the introduction of functional groups in the starch molecules increasing the binding force of the starch granules.

Least gelation concentration: The gelation properties of native acha starch and cross-linked sample are presented in table 3. The least gelation concentration increased from 6 % to 8 %. Cross-linking introduced bulky functional groups

during modification which minimized intermolecular interaction and caused electrostatic repulsion amongst the starch molecules thereby increasing least gelation concentration values. Gelation property of starch is a phase phenomenon resulting from aggregation of starch molecules.

Starch pasting properties: The pasting viscosity of native acha starch (NAS) and modified sample are presented on table 4. These are shown as viscosities (Cp) at room temperature using Brookfield Viscometer. The pasting viscosities of modified samples were reduced from 31.5 centipoise for native acha starch to 25.0 centipoise for cross-linked sample.

Starch granule morphology: The granule morphology of native acha starch (NAS) and modified sample are shown in figures 1. These morphologies were investigated using scanning electron microscopy at 15keV accelerating voltage and 2500 magnification each. It is obvious that modifications may not have destroyed the shape, appearance and structural arrangements of the starch. The starch granules retain their polygonal shape with sizes ranging from 6µm to 8.57µm.

IR-Study

The infrared (IR) spectra of native acha starch (NAS) and modified derivatives (XAS) are presented on Figs. 2 and 3 respectively. The infra red spectra have similar peaks, except for the additional peak at 1614 cm⁻¹ on cross-linked sample (Fig 3) which is ascribed to the ester carbonyl band of the citric acid cross-linked sample. These bands confirm that modifications of native acha starch were successfully carried out.

CONCLUSIONS

Chemical modifications of Acha starch by crosslinking was successfully carried out. Some physicochemical properties of the native starch (NAS) were altered upon modification. Chemical modifications enhanced emulsion capacity and least gelation concentration. Water and oil absorption declined. Rheological properties expressed as paste viscosities were also altered on modified sample. The viscosities of cross-linked sample were reduced. Potential applications of crosslinked acha starch include good emulsifying agent, starch thickened sauces, soups, paper binding and pharmaceutical drug carriers and disintegrants. Modifications improved stability and retrogradation.

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